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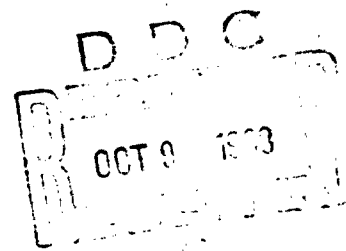
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## THE EFFECT OF X-RAYS ON MULTIPLICATION OF THE VACCINIA VIRUS IN TISSUE CULTURE

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The tissue culture method is widely used in radiobiology for studying the characteristics of the interaction of viruses with irradiated cells. However, the results of the investigations on the effect of ionizing radiation on the sensitivity of tissue culture cells to viruses are controversial. Thus, according to the data of some investigators [1-3], reproduction of viruses in irradiated cells is intensified; according to others [4-6], irradiation delays the developmental cycle of the virus, and in a number of papers [7-9] no difference is noted between the degree of virus reproduction in irradiated and non-irradiated tissue cultures. According to the sources in the literature presented, multiplication of the virus in irradiated tissue culture may depend on many factors: the type of culture, its age, radiation dose, interval between radiation and infection.

Previously, we have shown [10] that x-ray irradiation with a dose of 10 kr to a 72-hour cell culture of skin-muscle tissue of the chick embryo has no effect on the rate of multiplication of the vaccinia virus in it. The aim of the present work was to study the infectious and hemagglutinating properties of the vaccinia virus in skin-muscle tissue of the chick embryo irradiated with x-rays in a dose of 20 kr after 48 hours of cultivation.

### MATERIAL AND METHODS

Cultivation of the virus was carried out in trypsinized skin-muscle tissue of eight-nine-day chick embryos grown out in Earle's solution with 0.5 percent hydrolysate of lactalbumin and 10 percent beef blood serum. For the tissue cultures the vaccinia virus of series 183, obtained from the Scientific Research Institute of Epidemiology and Microbiology imeni I. I. Mechnikov, was used. One-tenth of a cc of the virus in the form of a 10 percent suspension of the chorioallantoic membrane of infected chick embryos was introduced into each test tube. After a 40-minute contact between the virus and the cells at room temperature the cells were washed three times with Hanks solution, to

eliminate the residue of non-adsorbed virus, and one cc of nutrient medium was added to the test tubes.

The tissue cultures were irradiated on an x-ray therapeutic RUM-11 apparatus with a voltage of 187 kv, current of 15 ma, dose rate of 515 r per minute, focal distance of 18 centimeters, with no filters; the radiation dose was 20 kr; exposure time, 38 minutes and 46 seconds. Test tubes containing the tissue culture were irradiated in cardboard boxes at room temperature; the non-irradiated cultures at this time were also left outside the incubator.

### FORMULATION OF THE EXPERIMENTS

Part of the 48-hour cultures were irradiated and then, after the completion of the irradiation, they were infected; another portion was simply infected. As a control irradiated as well as non-irradiated and uninfected tissue cultures were used. Seventy-two hours after infection, from every five experimental test tubes, samples of culture fluid were taken (0.5 cc each), mixed and kept in a refrigerator. The cell fraction in the same test tubes was washed free of the residues of culture fluid, washed with versene solution, centrifuged at 1000 rpm for 10 minutes, and the precipitate was again suspended in 2.5 cc of medium 199 containing two percent inactivated beef serum. The cells were destroyed by freezing and thawing three times. The liquid and the cell fraction of the viruses of the irradiated and non-irradiated tissue cultures were used for biological titration, performing the hemagglutination test as well as for subsequent passage through tissue culture. In both the first and second passages the experiments were performed three times. The statistical analysis was accomplished by the Ofvin method [11] and Student's Table.

### RESULTS

Observations of tissue cultures were made for 10 days. In the tissue culture exposed to irradiation alone a certain loss of cells and partial disappearance of syncytium were observed after three days. On the sixth day after irradiation lysis of the majority of cells was noted; the intact cells were enlarged, of triangular shape with signs of breakdown of the nuclear substance (Fig 1, a).

A visible cytopathogenic effect of the virus appeared 24 hours after infection: thinning of the monolayer, shriveling of the cells and the appearance of symplasts (Fig 1, b). Seventy-two hours after infection the tissue culture was represented by round cells deprived of nuclei. When the two factors acted on the tissue culture -- x-rays and the

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Fig 1. Tissue Culture of Chick Embryo. a. Six days after irradiation; b. 24 hours after infection with the vaccinia virus (magnification, 120 x).

virus -- the morphological changes in it were more marked. Thus, 72 hours after irradiation and infection the tissue consisted mainly of an accumulation of fragments of destroyed cells (Fig 2a). On the sixth day of cultivation the control tissue culture maintained its usual appearance (Fig 2b).

For a comparative study of the rate of accumulation of the infectious virus in irradiated and non-irradiated tissue cultures, the liquid and cellular fractions of it were titrated on chick embryos and on rabbits by the Groth method. As is evident from the Table data, in the first and second passages the virus titers in the liquid fraction of the irradiated tissue culture were higher than in the non-irradiated culture. The difference between the virus titers, expressed in log LD<sub>50</sub> for chick embryos, was statistically significant. Data presented in the Table show that the cell fractions of a tissue culture 72 hours after infection contain a very small quantity of the virus; therefore, it was impossible by the methods of titration used to establish a difference between the content of the virus in the cell fractions of the irradiated and

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Fig 2. Tissue Culture of Chick Embryo. a. 72 hours after irradiation and infection with the vaccinia virus; b. Normal tissue culture after six days of cultivation (magnification, 120x).

## Comparative Study of Infectious and Hemagglutinating Properties of the Vaccinia Virus in Irradiated and Non-Irradiated Tissue Culture

Tissue culture		Infectivity titers of cultured viruses				Virus titers in the hemagglutination test	
Type	Fraction	First passage		Second passage		Passages	
		In chick embryos in log LD <sub>50</sub>	In rabbits by the Groth method (in dilutions)	In chick embryos in log LD <sub>50</sub>	In rabbits by the Groth method (in dilutions)	I	II
Non-irradiated	Liquid	1.46 ± 0.11	10 <sup>-2</sup>	1.40 ± 0.11	10 <sup>-1</sup>	1:16	1:8
	Cellular	*	Undiluted	*	Undiluted	0	0
Irradiated	Liquid	2.40 ± 0.00	10 <sup>-2</sup>	2.45 ± 0.12	10 <sup>-1</sup>	1:16	1:8
	Cellular	*	Undiluted	*	*	0	0

\*No death of chick embryos or formation of an infiltrate in rabbits after injection of the original infectious material. 0 -- absence of hemagglutinins in the initial infectious material.

non-irradiated tissue cultures. The data also attest to the fact that irradiation has no influence on the accumulation of hemagglutinins in the liquid tissue culture fraction; in the cell fractions no hemagglutinins were found even in the initial material.

Thus, a somewhat greater accumulation of infectious virus in the liquid fraction of irradiated culture by comparison with non-irradiated virus 72 hours after infection was found. The data obtained permit us to believe that x-ray irradiation of a 48-hour culture of chick fibroblasts with a dose of 20 kr, performed directly before infection, increases the sensitivity of the cells to the vaccinia virus. Increase of the sensitivity of irradiated cells is proved by the great virus yield as well as by morphological observations indicating a more pronounced destruction of irradiated cells than non-irradiated cells.

### CONCLUSIONS

1. X-ray irradiation of a 48-hour skin-muscle tissue culture of a chick embryo with a dose of 20 kr causes an increase in the infectivity titer of the vaccinia virus in the liquid fraction of the tissue culture 72 hours after infection.
2. No difference is noted between the accumulation of hemagglutinins in the liquid fractions of irradiated and non-irradiated chick embryo tissue culture 72 hours after infection.

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